DISCOVERY

58(321), September 2022

Identification of key genes for predicting diabetic retinopathy prognosis by integrated computational analysis

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To Cite:
Odeigah LO, Agede OA, Obalowu IA, Mutalub YB, Olarinoye JK, Ambrose GO. Identification of key genes for predicting diabetic retinopathy prognosis by integrated computational analysis. *Discovery*, 2022, 58(321), 1031-1040

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Peer-Review History
Received: 20 July 2022
Reviewed & Revised: 23/July/2022 to 21/August/2022
Accepted: 24 August 2022
Published: September 2022

Peer-Review Model
External peer-review was done through double-blind method.

ABSTRACT

Diabetic retinopathy is a life-threatening disease with a poor prognosis. Therefore, it is necessary to identify molecular prospective biomarkers for diabetic retinopathy. The present study aims to identify prospective key genes that can be used to predict the prognosis of patients with diabetic retinopathy. GEO2R software was used to analyze the GEO dataset, which was downloaded from the Gene Expression Omnibus (GEO) database, in order to identify differentially expressed genes (DEGs). For these DEGs, we performed Functional analysis and pathway enrichment using the Database for visualization, Annotation and Integrated Discovery tool. A total of 5 DEGs were spotted in the GEO dataset including only genes up-regulated in diabetic retinopathy compared to normal tissues. The protein-protein interaction (PPI) network consisted of 5 nodes and 25 edges. In the PPI network, COX2, UQCRC2, TUFM, ABCCB6 and ND3 had a large number of interactions. The over-expressed DEGs were remarkably enriched in the biological processes "respiratory electron transport chain", "oxidative phosphorylation", "mitochondrial organization" and "formation of metabolites and energy precursors", and the cellular components "ribosomal subunit", "organelle". membrane'.", "mitochondrion" and "cytoplasm" while in molecular function "active transmembrane activity driven by oxidation" and "redox activity". In summary, five (5) classes were identified by comprehensive bioinformatics analysis. These key genes can be used as prognostic biomarkers to predict patients with diabetic retinopathy and thus may represent new therapeutic targets for diabetic retinopathy.

Keywords: DEGs, diabetic retinopathy, biomarkers, prognosis

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease that affects the metabolism of carbohydrates, protein and fat and is caused by the depletion of insulin-producing cells in the pancreas or a decrease in the sensitivity of tissues to

insulin, proceeding to an increase in the level of glucose in the blood and high mortality associated with its complications such as retinal damage associated with diabetes [1, 2].

Diabetic retinopathy (DR), a plausibly blinding ocular complication of diabetes [3] and also one of the microcirculatory lesions of the general diabetic disease, which can be caused by changes in vessel walls, changes in blood flow, or changes in platelets. [4]. DR has three main stages (early and severe non-proliferative, proliferative diabetic retinopathy and diabetic macular edema) [4]. From a public health View-point, early detection and secondary intervention are essential, as vision loss from DR can usually be averted with early effective treatment. One of the most challenging problems facing ophthalmology research is DR. As its prevalence has increased worldwide, today it is among the most frequent causes of blindness, [5-8].

Many changes occur in retinal vessels with DR long before fundus examination disclose any pathologic changes. Long-term diabetes leads to an early and progressive loss of retinal pericytes and endothelial cells, resulting in microaneurysms [9]. In addition, increased leukostasis and changes in platelet-fibrin clots in the retinal capillaries contribute significantly to retinal ischemia and vascular occlusion. Chronic hypoxia therefore induces angiogenic growth factors such as vascular permeability factor (VPF) that eventually contribute to the retinal neovascularization connected with proliferative DR (PDR) [10]. DR is known to act in conformity with the classic course of disease progression from No-DR → no-PDR → PDR. However, not every patient progresses through this classic paradigm. For example, some patients develop mild NPDR after 8-10 years without DR. Of these people, about 50% of the people with T1D generate PDR while about 20% of those with type 2 diabetes generate PDR. After four years of moderate to severe NPDR, the prevalence of PDR is 11% and the prevalence of severe visual loss is 7.2% [11].

Candidate gene studies are clinical and preclinical studies in which a gene is identified as potentially involved in disease pathogenesis based on the expression of proteins already identified in the disease state. A 2009 meta-analysis examined 34 genetic variants known to be associated with the pathogenesis of DR and found that the aldo-keto reductase gene (ALR) family 1 member B, (AKR1B1) had the highest number of polymorphisms associated with DR without correlation of ethnicity. Other polymorphisms described to be remarkably connected with DR comprise NOS3, VEGFA, integrin subunit alpha 2 (ITGA2), and intercellular adhesion molecule 1 (ICAM1) [12]. Lately, Sharma et al. (2019) fashionably condensed candidate gene studies connected with DR in a review spotlighting 65 dissimilar genes [13]. These candidate genes are convoluted in a diversity of cellular processes, including glucose metabolism, inflammation, angiogenesis, and neurogenesis. These studies have generally produced inconsistent and controversial results [14]. Consequently, this study's objective is understanding the basis of such differences and thereby identify the key genes responsible for the prognosis of diabetic retinopathy.

2. MATERIALS AND METHODS

2.1. Microarray datasets

The GEO database (http://www.ncbi.nlm.nih.gov/geo) is a general functioning genomics database that contains a variety of data, including data derived from microarrays and next-generation sequencing. The GEO database was searched using the following keywords: ("diabetic retinopathy" [MeSH terms] OR retinopathy [all fields])) AND "Homo sapiens"[porgn] AND ("gse"[filter] AND "profile expression per matrix" [filter] AND "feature name texture" [filter]. A total of 9 datasets were pinpointed for additional analysis after a keyword-based meta-analysis, the option basis for these datasets were as follows: a) incorporated datasets must comprise paired diabetic retinopathy and normal control tissues and ii) sufficient clinical information must be available to perform the analysis. Following the selection criteria, a gene expression profile was collected, which is GSE53257 [15]. The microarray data of GSE191210 were obtained from the GEO database. GSE53257 was based on the Homo sapiens conventional Human 8x15k Array system. The GSE53257 dataset was divided into 3 groups, which were group A consisting of non-diabetic (normal) patients, group B consisting of DR patients, and group C consisting of DM patients (Figure 1).

2.2 Data pre-processing and identification of Differentially Expressed Genes (DEGs) in diabetic retinopathy

Table array files of GSE53257 were downloaded from the GEO database. Prior to analysis, probes in each data set were converted to standard gene symbols. Normalization of the datasets was applied based on robust multi-array averaging in R software, version 4.0.0 (www.R-project.org/), and normalization was performed separately on each gene expression dataset. The GEO2R platform is a comprehensive software for visualization and statistical analysis of microarray gene expression data [16]. In the present study, DEGs from each dataset were pinpointed using GEO2R software. A P value of 0.05 was set as the cut-off criterion. A heatmap and volcano plot of DEGs were then generated from the dataset using the ggplo2 package in R software.

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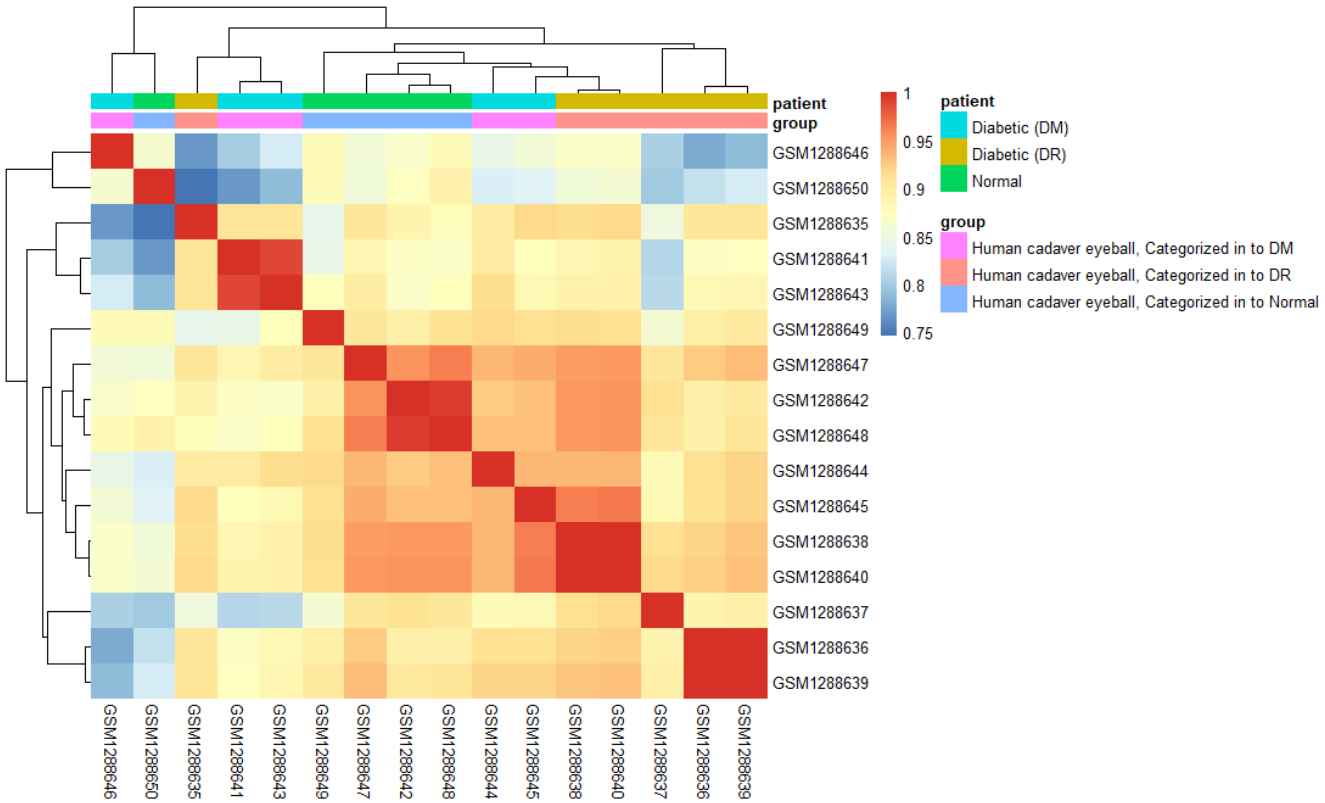

Figure 1: The structure of GSE53257 dataset

2.3 Functional and pathway enrichment analysis

A frequent method used for large-scale functional studies of transcriptomic data and genomic data analysis is Gene ontology (GO) analysis [17]. The Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/pathway) is a database for the structured evaluation of gene functions [18]. The Integrated Annotation and Discovery Visualization Database (DAVID; https://david.ncifcrf.gov) is a tool used to systematically determine biological significance in large lists of genes or proteins [9]. In the present study, GO function and KEGG pathway enhancement analysis of the identified DEGs and genes in the significant sections were performed using DAVID. Terms with a P value <0.005 were considered significant.

2.4 Pathway analysis and protein-protein interaction analysis

An enrichment map is a network-based method for visualizing and interpreting gene array enrichment [20]. In order to extract interactions between significantly enriched signalling pathways, pathway intersection analysis was performed using the Enrichment Map tool (http://www.baderlab.org/Software/EnrichmentMap). A Benjamini-Hochberg adjusted P value <0.05 was considered silver. The Jaccard coefficient and the overlap coefficient are two indices for examining the similarity between groups of samples.

STRING version 11.0 is a protein-protein interaction (PPI) database covering 5090 organisms. The STRING database is run to access protein-protein interactions, including direct (physical) and indirect (functional) connections [21]. To assess the relationship between the DEGs identified in this study, STRING was used and a PPI network was obtained through function and pathway enrichment analysis. This is considered statistically significant at $p < 0.05$.

3. RESULTS

3.1. Identification of DEGs

Incidence and mortality rates of diabetic retinopathy remain high regardless of advances in surgical and medical treatments for it, [21]. Successful screening and risk reduction techniques for diabetic retinopathy are crucial to lessen the occurrence of diabetic retinopathy. Understanding the etiology and progression mechanisms of diabetic retinopathy is essential to improve the survival rate of patients with diabetic retinopathy and prevent the onset of the disease. Recently, rapidly developed microarray technology

has been widely applied to make an analogy between gene expression levels and used to predict disease progression, for accurate diagnosis and prognosis assessment [22-23]. In the current study, a total of 5 DEG from the GSE53257 dataset were identified in DR tissues compared to normal tissues, all of which are up-regulated genes (Fig. 1). Volcano plots were created that show the distribution of these DEGs in the data set (Figure 2).

Table 1: DEGs based GSE53257 datasets comparing DR and normal tissues

ID	GeneSymbol	log2(fold change)	-log10(Pvalue)
A_2012_AEH0443	ISCU	0.2905246	3.79839
A_2012_AEH0709	SLC25A19	0.5932271	4.05699
A_2012_AEH0766	ACYP2	0.5068344	4.13331
A_2012_AEH0874	NIPSNAP3A	0.387464	4.45936
A_23_P53126	LMO2	0.9847524	6.211619

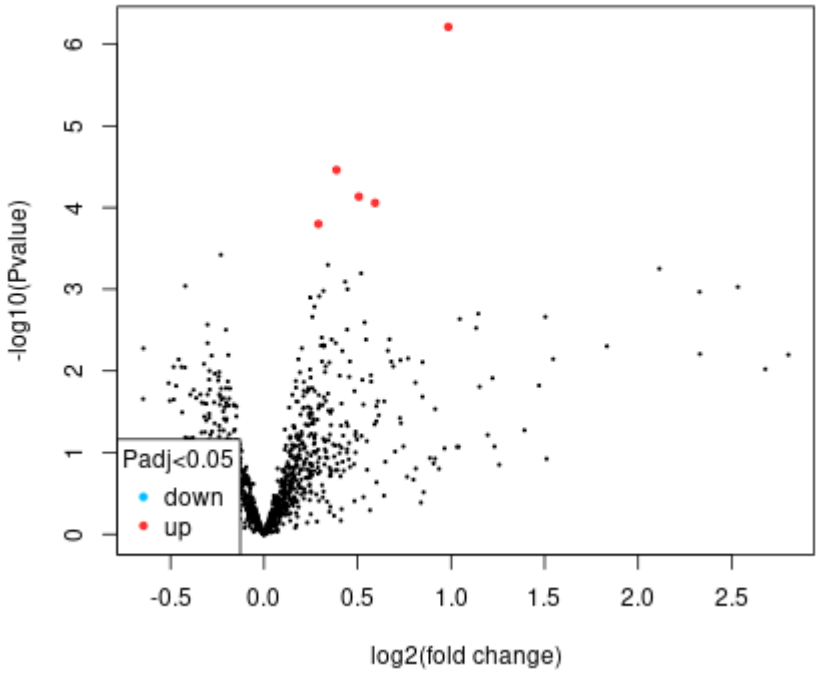

Figure 2: Volcano plot exhibiting expression data of diabetic retinopathy and normal tissues in the microarray profiles of GSE53257

3.2 Functional and pathway enrichment analysis

In order to fully understand the biological roles of these DEGs in diabetic retinopathy, DAVID was used to identify the GO functions and pathways in which they are involved. The over-expressed DEGs were remarkably enriched in the biological processes (Figure 3) "respiratory electron transport chain", "oxidative phosphorylation", "mitochondria organization" and "metabolite and energy precursor formation" and cellular components (Figure 4). "Ribosomal subunit", "membrane organelle", "mitochondrion" and "cytoplasm", while in molecular function "transmembrane activity prevents redox" and "redox activity" (Figure 5).

Mitochondrial dysfunction may lead to the development of diabetes mellitus with the associated risk of developing diabetic retinopathy or contribute directly to the metabolic dysregulation of diabetes and thus increase the risk of late complications of diabetes including retinopathy [24]. Diabetes in mitochondrial diseases can lead to the buildout of vision-threatening retinopathy, but visual s is sensitivity often decreased incidental to neurological deficiency resulting from mitochondrial dysfunction [25]. The relationship betwixt mitochondrial disease and diabetic retinopathy may be determined by epigenetics where environmental factors alter the expression of regulatory proteins encoding reactive oxygen species scavenging [26]. Mutant ISCU, one of the genes upregulated in this study, was found in patients with hereditary mitochondrial myopathy with exercise intolerance and lactic acidosis. This disease is a result of ISCU deficiency corresponding to the deficiency of mitochondrial iron-sulfur proteins and damage to oxidative muscle metabolism [27-28].

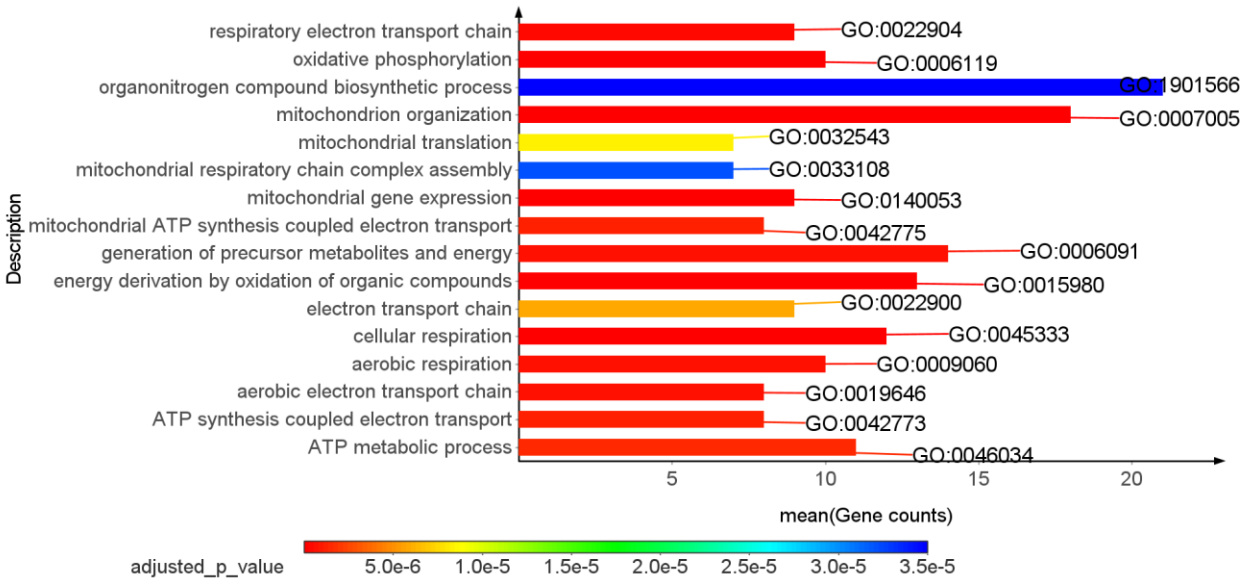

Figure 3: Gene ontology (GO) analysis of differentially expressed genes in diabetic retinopathy (Biological process)

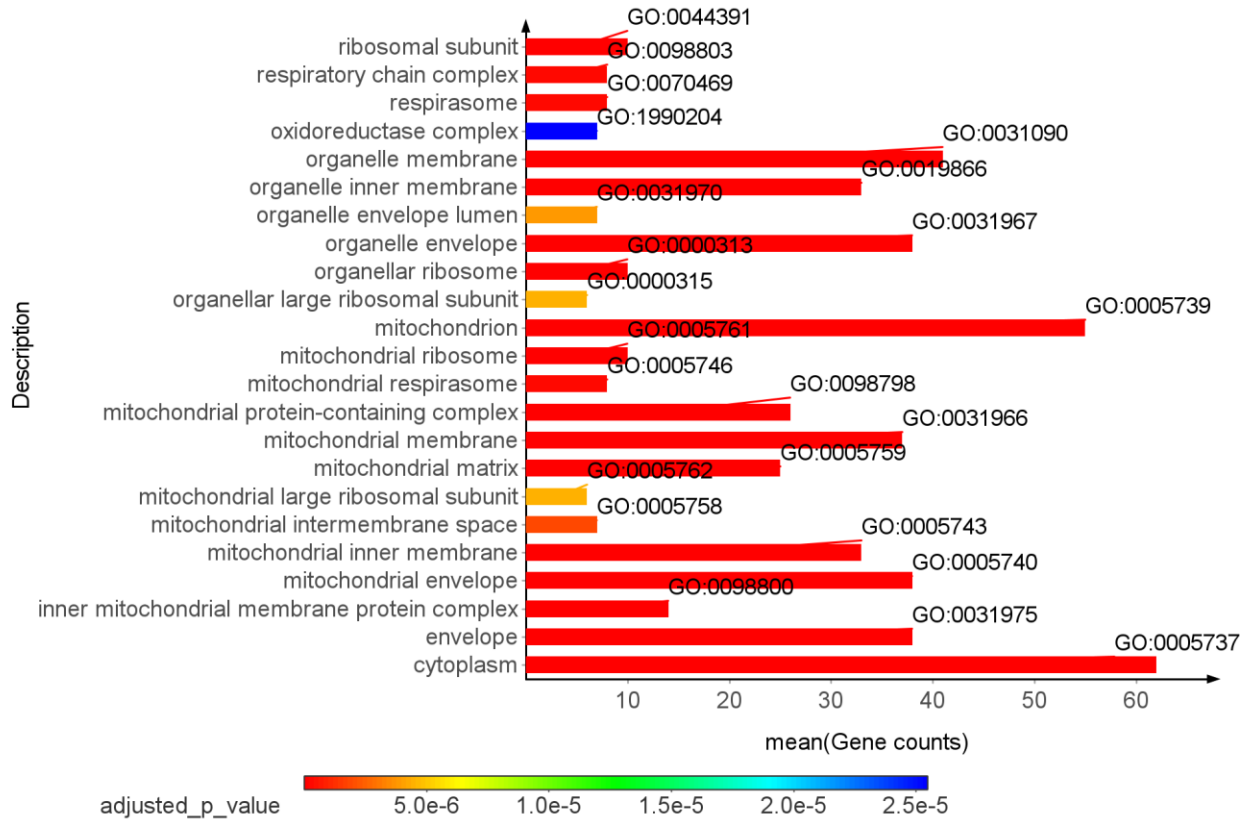

Figure 4: Gene ontology (GO) analysis of differentially expressed genes in diabetic retinopathy (Cellular components)

ISSN 2278–5469 EISSN 2278–5450 I OPEN ACCESS

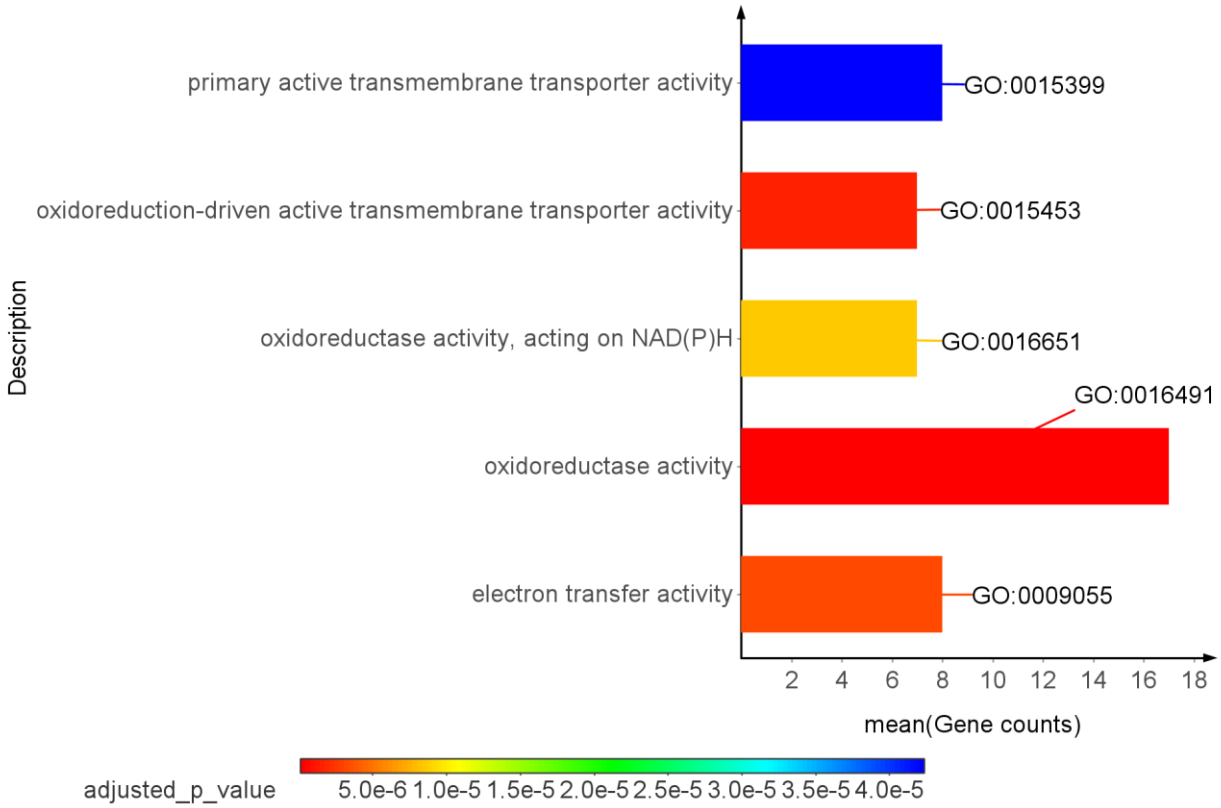

Figure 5: Gene ontology (GO) analysis of distinctively expressed genes in diabetic retinopathy (Molecular function)

According to the differentially expressed genes, the results of KEGG analysis showed that increased serum lactate, metabolic acidosis and lactic acidosis are involved in diabetic retinopathy, whereas mitochondrial diseases, leukoencephalopathy and leukodystrophy are associated with the disease (Figure 6).

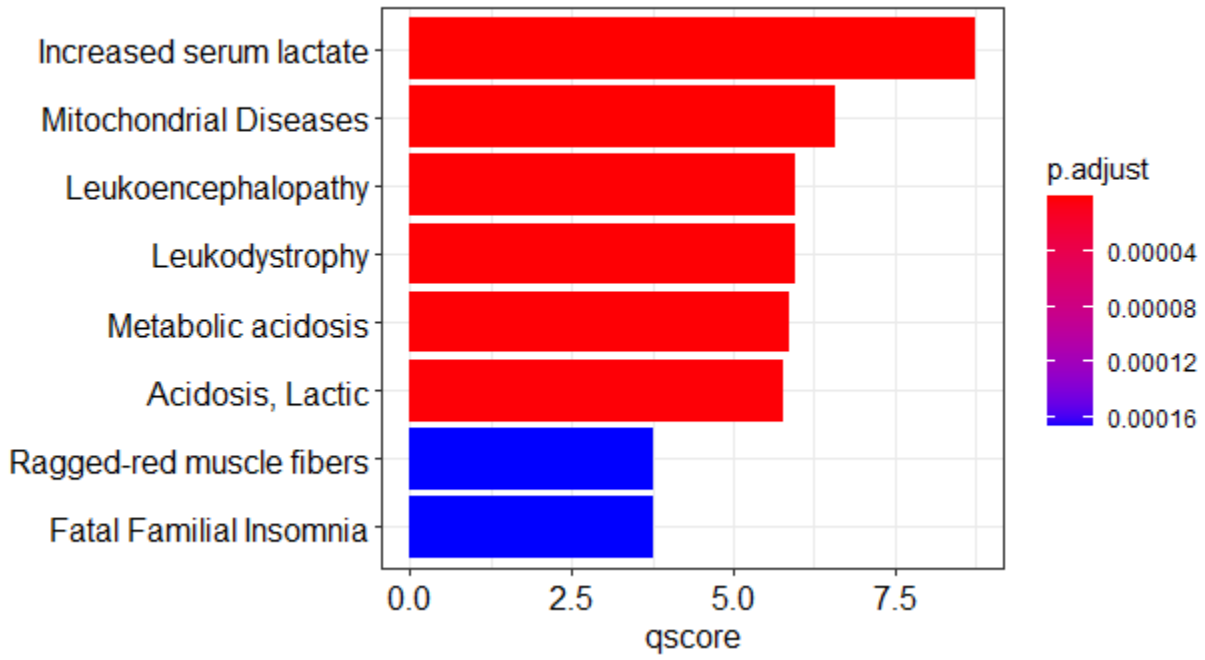

Figure 6: Kyoto Encyclopedia of Genes and Genomes pathway analysis of distinctively expressed genes in diabetic retinopathy

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The highly homologous NIPSNAP3 and NIPSNAP4 genes, in the company of 87% amino acid specification, are projections of the NIPSNAP family with potential roles in vesicle trafficking [29]. NIPSNAP3 and NIPSNAP4 mRNA protein have been detected in various tissues and cells to varying degrees. Many interrelated molecular pathways are involved in the development of DR. Microvesicles (MVs) are cell membrane vesicles, which bear many biological molecules, such as mRNA, microRNA, membrane receptors, membrane lipids, transcription factors, and other proteins. They may be entailed in intercellular transmission that can advance inflammation, angiogenesis, and coagulation. Recent studies have shown that changes in the number and composition of MVs may reflect the pathological conditions of DR [30].

LMO2, one of the significantly expressed genes in this study, is only a LIM domain protein. LIM domains were originally thought to bear Fe-S centers, but the demonstration of zinc in proteins [31]. LMO2 is expressed in the blood vessels of many tumor types [32] and fulfills its normal angiogenic role [33] in an undefined manner. Increased angiogenesis is implicated in various clinical conditions, including eye diseases such as proliferative diabetic retinopathy, and has also been found to occur due to the inflammatory response in ailment such as rheumatoid arthritis and intestinal diseases [34]. Therefore, LMO2 inhibition could be beneficial for proliferative diabetic retinopathy, but also for other diseases that have angiogenic processes.

3.3 Protein-protein interaction analysis

To efficiently analyse protein-protein interaction in our study, a subset of proteins with significant differential expression (FC > 1.5, $p < 0.05$) was selected (Figure 7). The PPI network comprised of 5 nodes and 25 edges (Figure 8). In the PPI network, COX2, UQCRC2, TUFM, ABCCB6 and ND3 had a large number of interactions (as shown in size captions). Therefore, in further studies for the treatment of diabetic retinopathy, they were considered to be the prospective proteins.

Intravitreal neovascular diseases, such as ischemic retinopathy, are the leading source of blindness. Because inflammatory mechanisms affect vitreous neovascularization and cyclooxygenase (COX)-2 fosters tumor angiogenesis, a previous study examined the role of COX-2 in proliferative ischemic retinopathy [35]. They described in their findings that COX-2 is generated in retinal astrocytes in human diabetic retinopathy, in mouse and rat proliferative ischemic retinopathy models in vivo, and in hypoxic astrocytes in vitro [35].

The results of the present study may have clinical relevance for human diabetic retinopathy. However, the present study presented some limitations. Since the potential roles of SLC25A19 and ACYP2 in human diabetic retinopathy remain unknown, future investigations will determine the effects of these genes in human diabetic retinopathy using experimental in vivo and in vitro.

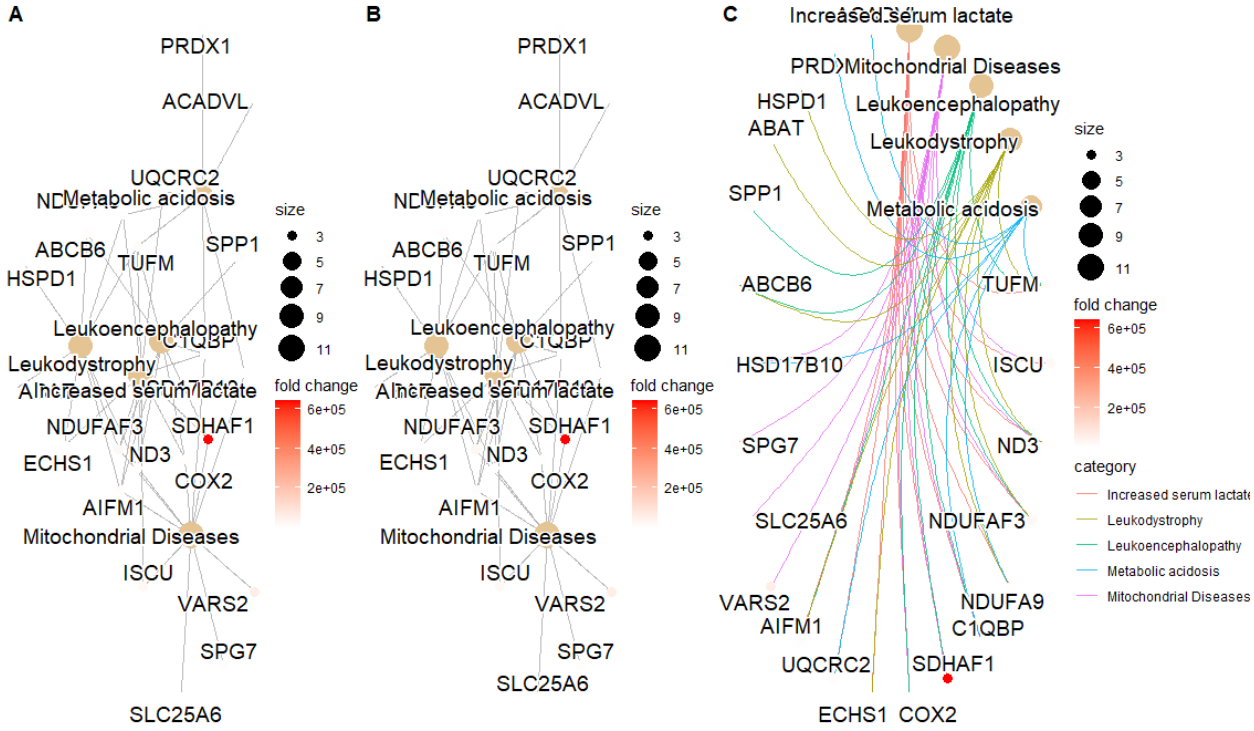

Figure 7: PPI **Network plot of enriched terms**

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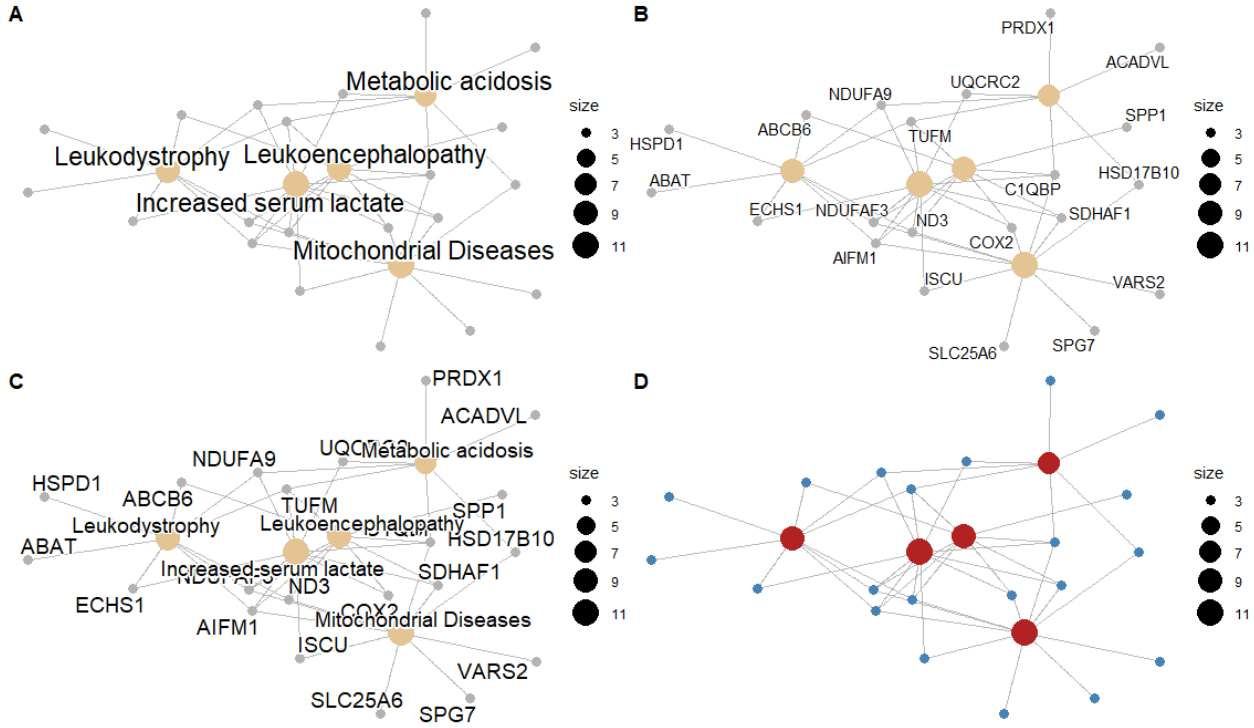

Figure 8: Labelling nodes by selected subset. gene category (A), gene name (B), both gene category and gene name (C) and not to label at all (D).

4. CONCLUSION

In conclusion, through GEO data analyses, five DEGs (ISCU, *NIPSNAP3*, LMO2, SLC25A19 and ACYP2) were reconized as being remarkably associated with patients with diabetic retinopathy. These five genes may serve as novel independent prognostic biomarkers that could be used to predict the clinical outcomes of patients with diabetic retinopathy. However, further investigations using cell lines or animal models are required in order to determine the underlying mechanisms of these five genes and their roles in the prognosis of diabetic retinopathy.

Funding

This study has not received any external funding.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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